Features of Hepatitis E Virus Infection in Japan

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Abstract

Hepatitis E virus (HEV) is a major cause of acute hepatitis in many developing countries. HEV is transmitted principally by the fecal-oral route, and water-borne epidemics are characteristic of hepatitis E. Recently, there is growing consensus that HEV-associated hepatitis also occurs among individuals in industrialized nations who had no history of travel to endemic areas. Zoonotic spread of HEV has been suggested as human and swine HEV strains are closely related genetically and experimental cross-species infection of swine HEV to a chimpanzee and that of human HEV to swine have been demonstrated. This review describes the clinical, epidemiological and virological characteristics of domestic HEV infection in Japan, the genetic relatedness of Japanese human and swine HEV strains, and possible modes of HEV transmission, emphasizing that HEV should be considered in the diagnosis of acute or fulminant hepatitis of non-A, non-B, non-C etiology, even in patients who have not traveled abroad.

(Key words: hepatitis E, hepatitis E virus, zoonosis, genotype, phylogenetic analysis, fulminant hepatitis)

Introduction

Hepatitis E virus (HEV) is a major cause of acute hepatitis in many developing countries in Asia, Africa and Latin America wherein hepatitis E is an important public health concern (1). HEV is an unclassified, small, non-enveloped RNA virus that is transmitted principally by the fecal-oral route. It can cause large water-borne epidemics of disease, although most infections are sporadic. Recent studies have documented that sporadic acute hepatitis E also occurs among individuals in industrialized countries with no history of travel to areas endemic for HEV (1–3).

The genome of HEV is a single-stranded, positive-sense RNA of approximately 7.2 kilobases in length, and contains a short 5' untranslated region (UTR), three open reading frames (ORFs: ORF1, ORF2 and ORF3), and a short 3' UTR terminated by a poly(A) tract (4, 5). Only one serotype of HEV is recognized, but at least four major genotypes (I–IV) have been identified (2). The majority of HEV infections in several countries in Asia and Africa are caused by genotype I, and the majority in Mexico and Nigeria are caused by genotype II, while only isolated cases of infection with HEV of genotype III or IV have been described in the United States, European countries, Argentina, China, Taiwan, and Vietnam (6–16).

Zoonotic spread of HEV has been suggested as human and swine HEV strains are closely related (7, 8, 17–22) and experimental cross-species infection of swine HEV to non-human primates such as a chimpanzee, rhesus monkeys, and cynomolgus macaques and that of human HEV to swine have been demonstrated (17, 20, 23). Recently, it was reported that swine veterinarians in the United States (24) and other pig handlers in China, Taiwan, and Thailand (7, 25) are at increased risk for HEV infection, suggesting that swine are animal reservoirs of HEV infection.

In Japan, hepatitis E was rarely reported until recently and most of the observed cases of hepatitis E had been regarded as imported cases of hepatitis (26, 27). Recently, however, a seroepidemiological study revealed that approximately 2–14% of the general population of Japan are positive for the immunoglobulin G (IgG) class of antibodies against HEV (anti-HEV IgG) (28, 29). In 2001, a presumably Japan-indigenous HEV strain (JRA1) was first isolated from a Japanese patient with acute hepatitis of non-A, non-B, non-C (non-ABC) etiology who had never been abroad (30), and swine HEV strains with high similarity to the JRA1 isolate have been isolated from farm pigs in Japan (31), suggesting circulation of indigenous HEV strains in Japan.

Characteristics of Domestically Infected Hepatitis E in Japan

In our recent study, 11 (13%) of 87 Japanese patients who had previously been diagnosed with sporadic acute hepatitis of non-ABC etiology were found to be infected with HEV of genotype III or IV (32). Interestingly, among sporadic acute
hepatitis cases of non-ABC etiology, HEV-associated hepatitis was significantly associated with males, higher age (≥40 years) and living in the northern part of Japan (Hokkaido and Iwate) (p<0.01, p<0.005, p<0.05, respectively). When restricted to male patients, patients aged ≥40 years or patients living in Hokkaido and Iwate, the prevalence of hepatitis E among sporadic acute hepatitis cases of non-ABC etiology was estimated to be 9/38 (24%), 11/48 (23%) or 8/38 (21%), respectively. Furthermore, the 11 patients with hepatitis E had markedly elevated serum transaminase levels [alanine aminotransferase (ALT), 914 to 4,850 IU/l; aspartate aminotransferase (AST), 539 to 5,931 IU/l]. Ten of the 11 patients had an elevated total bilirubin level (1.5 to 24 mg/dl) with protracted jaundice in two patients.

Although HEV induces a self-resolving hepatitis, it may be fatal, especially in pregnant women in developing countries (1). HEV infection is one of the major causes of fulminating hepatitis in India and Chad (28–44%) (33–35). We reported three male patients (60, 61 or 65 years of age) with fulminating hepatitis E who were infected with HEV of genotype III or IV (36). Furthermore, two of 10 patients with hepatitis E in Hokkaido were diagnosed as having fulminating hepatitis; the two cases were male, were 58 or 64 years of age, and were infected with HEV of genotype IV (37). These five cases of fulminating hepatitis E died 16–105 days after the onset of hepatitis E (Table 1). Recently, Ohnishi et al (38) reported two cases of fulminating hepatitis E in Hokkaido; one of them was a 34-year-old woman who was infected with a genotype III HEV and died after liver transplantation, and the other was a 51-year-old man who was infected with a genotype IV HEV and survived.

To date, 46 Japanese patients have been diagnosed with hepatitis E in our laboratory based on the presence of both the immunoglobulin M (IgM) class of anti-HEV (anti-HEV IgM) and HEV RNA (32, 36, 37, 39–44 and unpublished observations) in their sera that had been obtained at admission. They had no history of travel to endemic areas nor contact with travelers abroad or foreigners. The clinical and epidemiological characteristics of hepatitis E in these 46 patients are summarized as follows: 1) the age of the patients ranged from 38 to 86 years with a mean age of 59.6 years; 2) 40 patients (87%) were male; 3) 6 (13%) of the 46 patients developed severe prolonged jaundice and 5 other patients (11%) contracted fulminating hepatitis as described above (Table 1); 4) the month of onset of hepatitis E was distributed almost equally over the year and there was no particular season when hepatitis E predominantly occurred; and 5) there was wide variation in the geographical distribution of hepatitis E with a higher prevalence in the northern part of Japan (Hokkaido Island and northern part of mainland Honshu). Therefore, in Japan, clinical HEV infection should be taken into consideration when confronted with patients with sporadic acute or fulminating hepatitis of non-ABC etiology, paying special attention to age, gender, and location of residence.

### Domestic Hepatitis E in 1982

The question of how long ago the ancestor of the presently domestic HEV strains made inroads into Japan is left unanswered. Although still preliminary, we have found a clue for the answer to this question (40). We analyzed stored serum samples from a 38-year-old man who had been admitted to a city hospital in Mito, Japan, in January 1982 for jaundice and general fatigue, and who was given a clinical diagnosis of sporadic acute hepatitis of non-A, non-B etiology at that time. A stored serum sample that had been obtained on admission was found to be positive for anti-HEV IgM and HEV RNA. He had never traveled outside Japan and had no contact with travelers to areas of endemicity. There had been no cases of hepatitis in his family.

The HEV isolate (HE-JO-1982) from the infected patient was close to known genotype III isolates, with 87.6–94.4% identity in a 412-nucleotide (nt) ORF2 sequence, and was most closely related to the JRA1 isolate of genotype III which is considered to be indigenous to Japan (30). These results indicate that a presumably indigenous HEV isolate was circulating in Japan even in the early 1980s. The finding that a domestic HEV strain(s) has been present in Japan for more than two decades needs to be taken into consideration in future epidemiological studies on clinical and subclinical HEV infection and for the proper diagnosis of hepatitis E in industrialized countries where HEV infection was believed to be non-endemic.

### Polyphyletic Strains of HEV Isolated from Japanese Patients

Multiple HEV strains of genotype III or IV have been isolated from Japanese patients with sporadic acute or fulminating hepatitis.
Figure 1. Phylogenetic tree constructed by the neighbor-joining method based on the partial nucleotide sequence of the ORF2 region (301 nucleotides; nt 6,037–6,337 of the HE-JA10 genome (AB089824)) of 90 human HEV isolates using avian HEV as an outgroup. In addition to 27 reported human HEV isolates of genotypes I–IV whose entire or nearly entire sequence is known (marked with asterisks), 63 reported isolates of genotypes III and IV whose partial sequence of 301 nucleotides is available were included for comparison with accession nos. in parentheses. For visual clarity, Japanese HEV isolates are indicated in bold type and the four clusters of III JP, IIIus, IIIsp or IVjp of Japanese HEV isolates are shaded.
hepatitis E (30, 32, 36, 37, 39–44). The 41 Japanese HEV isolates thus far reported were genetically heterogeneous and they segregated into four phylogenetic clusters within two major genotypes, III and IV. The four phylogenetic clusters have been tentatively designated as clusters IIIjp, IIIus, and IIIsp within genotype III and cluster IVjp within genotype IV (the letters ‘jp’ stand for “presumably Japan-indigenous”, the letters ‘us’ stand for “US-like”, and the letters ‘sp’ stand for “close to Spanish isolates”) (Fig. 1). Cluster IIIjp comprises 9 HEV isolates; cluster IIIus comprises 13 isolates; cluster IIIsp comprises a single isolate; and cluster IVjp comprises 18 isolates. Upon comparison with HEV isolates of non-Japan origin whose ORF2 sequence of 301 nt is known, the cluster IIIus isolates were closely related to the US1 and US2 isolates of U.S. origin (9, 17), with 91.4–95.3% nucleotide sequence identity, although the cluster IIIjp and cluster IIIsp isolates were only 82.7–87.7% similar to these US strains. The cluster IIIsp isolate of HEV-Sendai was most closely related to the Spanish HEV isolates (VH1 and VH2) (8) with 85.2–85.9% nucleotide identity, among the HEV isolates thus far identified. The HEV isolates of cluster IVjp belong to genotype IV, although they were clearly separate from 30 known genotype IV isolates from China and Taiwan, differing by 10.3–15.6% in the ORF2 sequence of 301 nt. Consequently, HEV isolates that segregated into at least four distinct clusters within two major genotypes (III and IV) are circulating in Japan and are responsible for sporadic acute or fulminating hepatitis E in Japan.

**Modes of HEV Transmission**

Although risk factors for acquiring sporadic acute or fulminating hepatitis E have not been recognized among individuals in industrialized countries, it is likely that the following forms of transmission occur in industrialized countries including Japan where sanitation systems are well established: (a) zoonotic infection, (b) transmission by food, (c) person-to-person transmission, (d) transmission via blood transfusion. The first two important modes of transmission will be described in detail below. It has been reported that during epidemics in HEV-endemic countries, person-to-person transmission of HEV plays a minor role in the spread of hepatitis, with an attack rate of <2.5% within households containing primary cases (45). Regarding our cases, the examined family members (spouses and children of patients) were exclusively negative for anti-HEV IgG, indicating that person-to-person transmission of HEV may be extremely rare, if at all, in Japan. Viremic blood donors are potentially able to cause transfusion-associated hepatitis E not only in areas of high endemicity (46), but also in non-endemic countries like Japan; a Japanese man in his 60s contracted hepatitis E from a blood transfusion that he had received during heart surgery at a city hospital in Hokkaido in 2002 (ABC Newsletter January 24, 2003; accessible at http://www.americasblood.org). Of note, one of our patients received blood transfusion two months before the onset of hepatitis E.

**Possible Zoonotic Infection of HEV**

Evidence is accumulating that hepatitis E is a zoonosis (1, 3, 47), and cross-species infection of HEV has been documented (17, 20, 23). Anti-HEV IgG has been detected among pigs in HEV-endemic countries such as China, Nepal, and Thailand as well as among pigs in non-endemic countries such as Australia, Canada, Germany, New Zealand, Taiwan, and the United States (7, 25, 48–50). In a recent study, we found a high prevalence of swine anti-HEV IgG among Japanese pigs of 2–6 months of age (58% or 144/2,500) (51). Thus, HEV is considered to be enzootic in pigs whether or not hepatitis E is common in the resident human population. Swine HEV strains were found in pigs from different geographic regions in the United States and they showed nucleotide sequence identity of 89–96% to human HEV strains of genotype III (US1 and US2) (18). Other studies performed in China, Spain, and Taiwan have also indicated that the HEVs isolated from pigs and humans in a particular geographical region are closely related genetically (7, 8, 21, 22).

A total of 144 swine HEV isolates were obtained from viremic pigs in 27 farms located throughout Japan, and phylogenetic analysis was performed based on the partial ORF2 sequence of 412 nt (31, 51 and unpublished observations). The 144 swine isolates belonged to genotype III or IV, and segregated into three clusters (IIIjp, IIIus, and IIIsp) within genotype III and one cluster (IVjp) within genotype IV, comparable with those of human HEV isolates of Japan origin (Table 2). When phylogenetic analysis including the swine and human HEV isolates was performed, the cluster IIIjp swine isolates grouped with 9 Japanese isolates of human HEV with the highest nucleotide identity of 98.8% between swJ18-2 and HE-JA9; cluster IIIus swine isolates grouped with 13 other Japanese isolates of human HEV with the highest nucleotide identity of 98.8% between swJw1-232 and HE-JA7; cluster IIIsp swine isolates grouped with a different Japanese isolate of human HEV with the highest nucleotide identity of 98.3% between swJ791 and HEV-Sendai; and cluster IVjp swine isolates grouped with the remaining 18 Japanese isolates of human HEV with the highest nucleotide identity of 100% between swJ13-1 and HE-JA1.

The full-length genomic sequences of the swJ13-1 and HE-JA1 isolates were determined (52). swJ13-1 was isolated from a 4-month-old farm pig born in Hokkaido in 2002, and HE-JA1 was recovered from a 55-year-old patient who lived in Hokkaido and contracted sporadic acute hepatitis E in 1997. Both isolates consisted of 7,240 nt excluding the poly(A) tail (Table 3), and contained three ORFs (ORF1, ORF2 and ORF3) that encoded proteins of 1,707, 674, and 114 amino acids. The overall nucleotide sequence identity between them was 99.0% and the deduced amino acid sequence identities of ORF1, ORF2, and ORF3 were 99.8%, 100%, and 100%, respectively. The observed high genomic similarity between swine and human HEV isolates in a
Table 2. Comparison of the 412-nucleotide ORF2 Sequence between Swine and Human HEV Isolates of Japan Origin

<table>
<thead>
<tr>
<th>HEV genotype (cluster)</th>
<th>No. of swine HEV isolates</th>
<th>No. of human HEV isolates</th>
<th>Identity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>III (IIIjp)</td>
<td>75</td>
<td>9</td>
<td>89.0–98.8</td>
</tr>
<tr>
<td>III (IIIus)</td>
<td>39</td>
<td>13</td>
<td>90.0–98.8</td>
</tr>
<tr>
<td>III (IIIsp)</td>
<td>18</td>
<td>1</td>
<td>89.3–98.3</td>
</tr>
<tr>
<td>IV (IVjp)</td>
<td>12</td>
<td>18</td>
<td>87.9–100*</td>
</tr>
</tbody>
</table>

*The swJ13-l and HE-JA1 isolates shared nucleotide sequence identity of 100% within the 412-nt ORF2 sequence and their full-length genomic sequences were determined (see Table 3).

Table 3. Comparison of the Nucleotide and Amino Acid Sequences between the swJ13-1 and HE-JA1 HEV Isolates

<table>
<thead>
<tr>
<th>Region</th>
<th>swJ13-1 (swine origin)</th>
<th>HE-JA1 (human origin)</th>
<th>Identity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total*</td>
<td>7,240 nt</td>
<td>7,240 nt</td>
<td>99.0</td>
</tr>
<tr>
<td>5'UTR</td>
<td>25 nt</td>
<td>25 nt</td>
<td>100</td>
</tr>
<tr>
<td>ORF1</td>
<td>5,121 nt</td>
<td>5,121 nt</td>
<td>98.9</td>
</tr>
<tr>
<td>amino acid</td>
<td>1,707 aa</td>
<td>1,707 aa</td>
<td>99.8</td>
</tr>
<tr>
<td>ORF2</td>
<td>2,022 nt</td>
<td>2,022 nt</td>
<td>99.4</td>
</tr>
<tr>
<td>amino acid</td>
<td>674 aa</td>
<td>674 aa</td>
<td>100</td>
</tr>
<tr>
<td>ORF3</td>
<td>342 nt</td>
<td>342 nt</td>
<td>99.7</td>
</tr>
<tr>
<td>amino acid</td>
<td>114 aa</td>
<td>114 aa</td>
<td>100</td>
</tr>
<tr>
<td>3'UTR*</td>
<td>70 nt</td>
<td>70 nt</td>
<td>98.6</td>
</tr>
</tbody>
</table>

*Excluding poly(A) tail.

restricted area of Japan further supports that sporadic hepatitis E in Japan is a zoonosis. However, no direct evidence of HEV infection from swine to humans in clinical cases of hepatitis E has been provided thus far. Among 46 patients with hepatitis E, only one patient who was a pig farmer had regular contact with pigs; the remaining 45 patients had no contact with pigs or other animals such as rats, mice, dogs, cows, sheep or goats that could also potentially serve as reservoirs of HEV (53–56).

Possible Transmission of HEV Via Food

As described by Smith (3), it is likely that foods can act as vehicles for transmission of HEV. The occurrence of acute HEV infection in an individual in Sicily after consumption of shellfish obtained from fecally contaminated waters, has been reported by Cacopardo et al (57). Similarly, Mechnik et al (58) reported an Israeli who acquired acute hepatitis E possibly by eating raw shellfish. Hartmann et al (59) attributed acute HEV infection in a Turkish international living in Germany to ethnic foods brought as a gift by a Turkish visitor. However, no case of hepatitis E has been unequivocally shown to be due to food consumption.

Between January 2001 and December 2002, a total of 38 patients with acute hepatitis were admitted to two city hospitals in Hokkaido where hepatitis E is most prevalent in Japan. Among the 38 patients, 10 patients contracted sporadic acute or fulminant hepatitis E, whereas only 3 patients developed acute self-limiting hepatitis A, indicating that hepatitis E is more prevalent than hepatitis A in Hokkaido (37). Interestingly, we found that 9 of the 10 patients with hepatitis E had ingested grilled, but occasionally undercooked, pig liver 2 weeks to 2 months before the disease onset (37). In contrast, none of the 22 patients with non-E acute hepatitis who were seen in the same city hospitals between 2001 and 2002, had a history of ingesting pig liver before the onset of the disease, the difference being statistically significant [9/10 vs. 0/22, p<0.0001 (Fisher’s exact test)]. Since the pig livers they ingested were not available for testing, we purchased 363 packages of raw pig livers as food from grocery stores near the residences of our subjects in Hokkaido and could show that a certain proportion of packaged pig livers as food (1.9% or 7/363) was contaminated with HEV. The incubation period for hepatitis E is considered to be 2 to 9 weeks, and therefore, we would like to speculate that inadequately cooked pig liver contaminated with HEV was the source of the patients’ infection. Our speculation is supported by the evidence that one genotype...
IV HEV isolate obtained from a package of pig liver had high nucleotide sequence identity of 97.8–100% with 10 HEV isolates of genotype IV recovered from patients living in Hokkaido, and that two genotype III HEV isolates from two distinct packages of pig liver had high nucleotide sequence identity of 96.6–100% with 5 human HEV isolates of genotype III obtained from patients who lived in Hokkaido. The extent of grilling may affect the risk of acquiring HEV infection via consumption of pig liver. In some patients’ families, the patient’s spouse ingested only well-cooked pig liver and did not contract HEV infection. Detection of HEV RNA by reverse transcription-polymerase chain reaction in raw pig livers in grocery stores does not necessarily mean that the swine HEV in the packaged pig livers is infectious. Further studies are needed to determine whether packaged pig livers as food not only in Hokkaido but also in other areas in Japan contain infectious virus and to investigate whether patients with hepatitis E in areas other than Hokkaido had a history of consuming pig liver as food before the onset of the illness. In the interim, however, since the presence of HEV among pig livers for sale as food is suggested, pig livers should be cooked well before ingestion to prevent the occurrence of possible food-borne hepatitis E in Japan.

Conclusions

HEV should be considered as a causative agent of sporadic acute or fulminant hepatitis of non-ABC etiology in Japan, paying special attention to the age, sex and location of residence of the patient. Transfusion-transmitted hepatitis E occurs, although only a single such case has been documented thus far in Hokkaido where hepatitis E is most prevalent in Japan and is more prevalent than hepatitis A. As suggested by the observed high genomic similarity between human and swine HEV isolates, patients’ dietary habits of ingesting pig liver and the presence of HEV in pig liver as food in Hokkaido, it seems likely that the domestic spread of clinical and subclinical HEV infection in Japan is via zoonosis and/or food. However, further clinical, epidemiological and virological studies are needed to elucidate the various and, possibly, region-dependent modes of HEV transmission and to prevent domestically infected hepatitis E in Japan.

Note added in proof: Hepatitis E after ingestion of the raw meat from a wild deer in Hyogo Prefecture (60), has recently been reported, indicating the presence of various modes of HEV transmission in Japan.

References

Domestic HEV Infection in Japan


